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GLUCOSE BIOSENSOR

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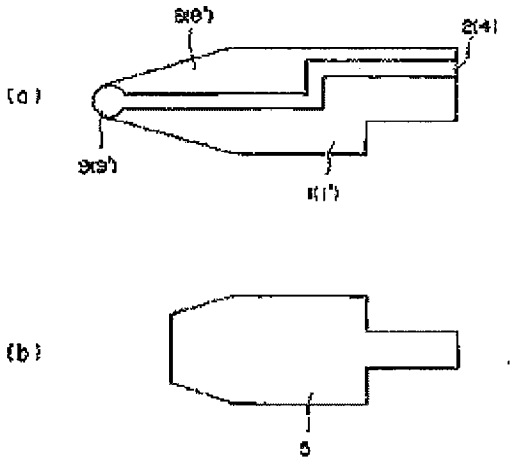
Abstract

Problem

To provide a glucose biosensor in which glucose oxidase has been affixed, with which easy production and measurement are possible, and which consequently is suitable as a disposable glucose biosensor.

Means of solution

An active electrode and counter electrode are disposed on the aforementioned glucose biosensor in an opposing surface structure. It is preferred that a spacer be placed between the substrate on which the active electrode is disposed and the substrate on which the counter electrode is disposed, that tapers be respectively formed at one end of each of the substrates, and that the leading edge of the active electrode or counter electrode be disposed at the tip of the taper.



Claims

1. A glucose biosensor, wherein an active electrode and counter electrode are disposed in a glucose biosensor, in which a glucose oxidase has been affixed, in an opposing surface structure.

2. The glucose biosensor disclosed in Claim 1, wherein a reference electrode is disposed on the active electrode side or the counter electrode side.

3. The glucose biosensor disclosed in Claim 1 or 2, wherein a spacer is placed between the substrate on which the active electrode is disposed and the substrate on which the counter electrode is disposed.

4. The glucose biosensor disclosed in Claim 3, wherein tapers are respectively formed at one end of each of the substrates, and the leading edge of the active electrode or counter electrode is disposed at tip of the taper.

Detailed explanation of the invention

[0001]

Pertinent technical field of the invention

This invention pertains to a glucose biosensor. In further detail, it pertains to a glucose biosensor in which glucose oxidase has been affixed.

[0002]

Prior art

In past glucose biosensors, in which glucose oxidase was affixed onto an active electrode, in addition to the active electrode, a counter electrode, or a counter electrode and a reference electrode were disposed in the same plane on a flat substrate. In glucose biosensors with this kind of electrode disposition, there are two methods of bringing the measurement sample in contact with the active electrode.

[0003]

The first method is a method in which a drop of the measurement sample is placed directly on the active electrode, but this method has the problem of requiring time and labor from when the sample is taken until the drop is placed. The second method is one that uses a structure in which a spacer with a groove is disposed over the electrode substrate and a cover with air holes is further disposed on top of that. This method has the advantage of not requiring time and labor to introduce the measurement sample directly to the active electrode, but is flawed in that it requires a troublesome process to prepare the elements, such as requiring the formation of air holes, etc.

[0004]

Objective of the invention

The objective of this invention is to provide a glucose biosensor in which glucose oxidase has been affixed, with which easy production and measurement are possible, and which consequently is suitable as a disposable glucose biosensor.

[0005]

Means to solve the problems

The objective of this invention is achieved by disposing the active electrode and counter electrode in an opposing surface structure in the aforementioned glucose biosensor.

[0006]

Conditions of embodiment of the invention

Figure 1(a) shows the structural elements, comprising an active electrode 2 and a reference electrode lead 3 formed on a substrate Figure 1(b), a counter electrode 4 formed on a substrate 1' Figure 1(c), and a double-sided adhesive spacer (approximately 100-500 μm-thick) 5 (c), and a plan view drawing of the element assembled from these is shown in Figure 2, and an elevation-view drawing thereof is shown in Figure 3.

[0007]

Glass, ceramic, paper, or biodegradable material (e.g., microbial polyester, etc.) is used as the substrate. The active electrode, counter electrode, and reference electrode lead can be formed by screen printing, vapor deposition, or sputtering, etc., from platinum, gold, or carbon, etc., while reference electrode 6 is formed by a method in which a silver electrode is formed on top of the reference electrode lead by screen printing, vapor deposition, or sputtering, etc., and then constant-current electrolyzed, or immersed into an aqueous solution of ferric chloride. And then said reference electrode is further coated and laminated by screen printing with silver chloride. The reference electrode can be disposed on either the active electrode substrate or on the counter electrode substrate, but it is preferable if it is disposed on the active electrode substrate. Further, a two-electrode structure without a reference electrode can be constructed in a similar manner.

[0008]

Glucose oxidase is generally affixed to the active electrode, but since glucose oxidase is soluble in the aqueous solution that is the measurement sample, and will react on the active electrode, it may also be affixed on the perimeter of the active electrode, or on the counter electrode and its perimeter.

[0009]

When affixing the glucose oxidase, preferably to the active electrode, not only can the glucose oxidase itself be affixed, but it may also be formed as a mixture layer in which at least one of an electron carrier (mediator) and albumin has been added, as listed in the examples below.

(1) Glucose oxidase layer

(2) Glucose oxidase – electron carrier mixture layer

(3) Glucose oxidase – albumin mixture layer

(4) Glucose oxidase – electron carrier -- albumin mixture layer

[0010]

Formation of the glucose oxidase layer (1) can be accomplished, e.g., by dissolving glucose oxidase (GOD), e.g., in the case of 165,800 units/g GOD, approximately 1-50 mg, preferably 5-30 mg, into 1 mL distilled water or buffer solution, and then dripping approximately 0.5-10 μL, preferably 1-3 μL, of this solution (GOD solution), or dripping it by spin-coating, and then drying it at room temperature to form a layer approximately 0.05-10 μm, preferably approximately 0.1-2 μm, thick.

[0011]

A similar formation method to the above can also be performed for mixture layers (2)–(4); however, a solution is used to which the various following constituents have been further added to the aqueous GOD solution.

Mixture layer (2): Using potassium ferricyanide or parabenzoquinone, etc. as the electron carrier, a solution is used to which approximately 1-100 mg, preferably approximately 30-60 mg, of potassium ferricyanide, or approximately 1-200 mg, preferably approximately 50-150 mg, of parabenzoquinone have been added

Mixture layer (3): A solution is used in which approximately 1-100 mg, preferably approximately 5-30 mg, of bovine serum albumin have been further added

Mixture layer (4): A solution is used in which the quantity of electron carrier used to form mixture layer (2) and the quantity of bovine serum albumin used to form mixture layer (3) have further been added

[0012]

The added electron carrier has the following action, and the addition of albumin provides measurement results with little variance relative to changes in the pH of the measured solution (aqueous glucose solution).

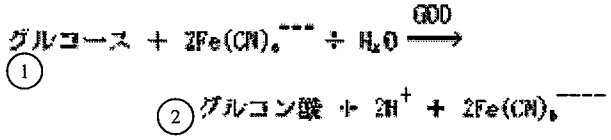
[0013]

A commonly known method for indirectly finding the glucose concentration is one in which gluconolactone is produced by oxidation of glucose in the presence of an enzyme by the action of GOD, and then the H_2O_2 produced at that time is oxidized on the active electrode and the oxidation current value at that time is measured. However, since the rate of the oxidation reaction

is dictated by the concentration of dissolved oxygen in the solution, in the case of undiluted samples, in which the measurement solution is not diluted with water, the glucose concentration will show a linear quantitation range of no more than approximately 100 mg/dL.

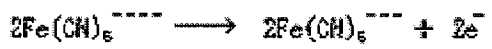
[0014]

Therefore, an electron carrier is used together with GOD instead of oxygen, whose concentration in the solution is limited. When the mediator is potassium ferricyanide $K_3Fe(CN)_6$, this reaction proceeds as follows.



Key: 1 Glucose
2 Gluconic acid

The ferricyanide ion produced at this time is oxidized by the active electrode to produce an oxidation current.



[0015]

In addition, when parabenzoquinone is used as the mediator instead of potassium ferricyanide, hydroquinone is produced by the reaction between glucose and parabenzoquinone in the presence of GOD, and the hydroquinone produced at this time is oxidized by the active electrode to produce an oxidation current, the value of which is measured.

Hydroquinone ——> Parabenzoquinone + $2H^+$ + $2e^-$

[0016]

Meanwhile, the counter electrode can be used without anything in particular affixed to it, and it may also be used with a layer of at least one of albumin or an electron carrier formed on it.

[0017]

Further, to smoothly bring the measurement sample solution in contact with the GOD that has been affixed, a surfactant, such as lecithin, etc., may be applied to the active electrode, the counter electrode, the area surrounding the active electrode, the area surrounding the counter electrode, the active electrode and the area surrounding it, or the counter electrode and the area

surrounding it, or some means can be employed, such as using a soaking promoter, such as felt cloth or filter paper, etc., wedged into the gap in the spacer.

[0018]

Measurement of the glucose concentration is performed by bringing approximately 0.1-10 μL aqueous glucose solution of a specified concentration into contact with the glucose biosensor produced in this fashion and allowing it to react for approximately 1-120 sec, then impressing a voltage of approximately 0.05-0.8V, preferably approximately 0.4-0.7V, and measuring the current value after impressing this for, e.g., 20 sec. Measurement is performed using a potentiogalvanostat and a function generator.

[0019]

When the aqueous glucose solution is brought in contact with the glucose biosensor, a taper 8 (8') is formed at one end of each substrate 1 (1'), and preferably the leading edge 9 (9') of the active electrode 2 (or the counter electrode 4) is disposed at the tip thereof, as shown in Figure 4. Namely, Figure 4(a) shows a structural element comprising an active electrode 2 (or counter electrode 4) formed on a substrate 1 (or 1') and Figure 4(b) a double-sided adhesive spacer 5, so that active electrode 2 and counter electrode 4 on the substrates 1, 1' can face inward and be formed into a single unit by means of the spacer 5. Since the spaced electrode gap is disposed in a pointed substrate taper in a sensor in this kind of embodiment, even trace amounts of measurement solution can be directly sampled, and consequently it is very suitable for rapidly bringing the sample in contact with the active electrode.

[0020]

Effect of the invention

By constituting the active electrode and counter electrode on opposing surfaces in a glucose biosensor, in which glucose oxidase has been affixed, simple production and measurement become possible, and consequently broad application of this kind of glucose biosensor can be anticipated since it can be effectively used as a disposable biosensor in which undiluted samples are the measured solution, as is the case in home health diagnosis (self-care), and especially for self-management of diabetes by measuring blood sugar or urine sugar, and in the prevention and early detection of diabetes, and for glucose management in the production of food products, etc.

[0021]

Example embodiment

This invention will be described below using an example embodiment.

[0022]

Embodiment

A carbon counter electrode, active electrode, and reference electrode lead were formed as shown in Figures 1-3 by screen printing to a thickness of 5 μm, on a polyethylene terephthalate film (0.25 mm-thick). Next, silver paste was screen printed to a thickness of 5 μm on top of the reference electrode lead, and then sintered to produce a silver electrode. The area of the silver electrode was dipped in 0.1 M hydrochloric acid and constant-current electrolyzed for 20 min at a current density of 0.6 mA/cm^2, to form silver chloride on its surface and to form a silver/silver chloride reference electrode. A potentiogalvanostat (Hokuto Denko Co., Ltd. HA501) was used for this constant-current electrolysis.

[0023]

1.5 μL of a mixed solution comprising 1 mL phosphoric acid buffer solution (pH 7.0), 10 mg glucose oxidase (165,800 units), and 48 mg potassium ferricyanide was then dripped on the active electrode of various electrodes with this kind of structure and then dried at room temperature to produce two types of glucose biosensors; A (without reference electrode) and B (with reference electrode).

[0024]

After introducing 5 μL of aqueous glucose solution of specified concentration into the sample inlet of the prepared glucose biosensors and allowing a reaction to proceed for 5 sec, a 0.6V voltage was impressed, and after being impressed for 20 sec, the current value was measured. A potentiogalvanostat (HA501) and function generator (Hokuto Denko Co., Ltd. HB-104) were used in this measurement.

[0025]

The measurement results (outputs) are shown in the following table and in the graph in Figure 5.

Table

Glucose Concentration (mg/dL)	A	B
0	0.5 μA	0 μA
50	1 μA	1 μA
100	2 μA	2 μA
250	5 μA	4 μA
500	9 μA	8 μA
800	14 μA	13 μA
1000	18 μA	16 μA

It can be understood from these results that linear quantitation can be obtained at glucose concentrations in the range of 0-1000 mg/dL. Further, each sensor was used to measure one sample and then discarded. In addition, at a glucose concentration of 100 mg/dL, the fluctuation coefficient, which shows the reproducibility for sensors (n = 10), was 3.6% for Sensor A and 3.5% for Sensor B.

Brief description of the figures

Figure 1 comprises various plan-view drawings of the element structural used in producing the glucose biosensor of this invention.

Figure 2 is a plan-view drawing of the assembled glucose biosensor.

Figure 3 is an elevation-view drawing of the assembled glucose biosensor.

Figure 4 comprises various plan-view drawings of the structural elements used to produce a preferred glucose biosensor of this invention.

Figure 5 is a quantitation graph that shows the relationship between the aqueous glucose solution concentration and output in the example embodiment.

Explanation of reference symbols

1 Substrate

2 Active electrode

3 Reference electrode lead

4 Counter electrode

5 Spacer
6 Reference electrode
7 Affixed glucose oxidase
8 Substrate taper
9 Electrode tip part

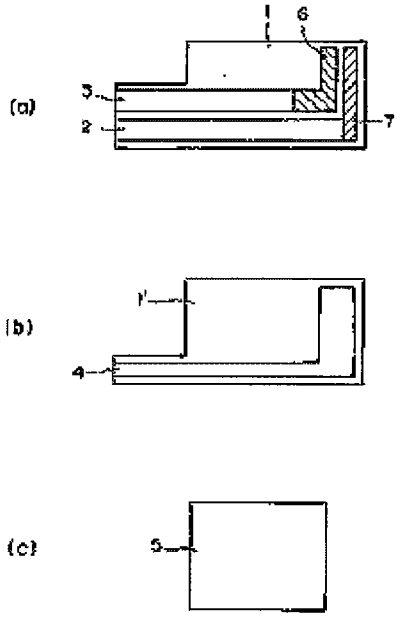


Figure 1

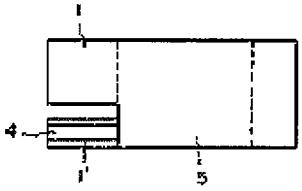


Figure 2

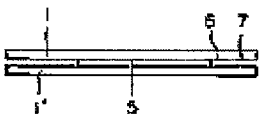


Figure 3

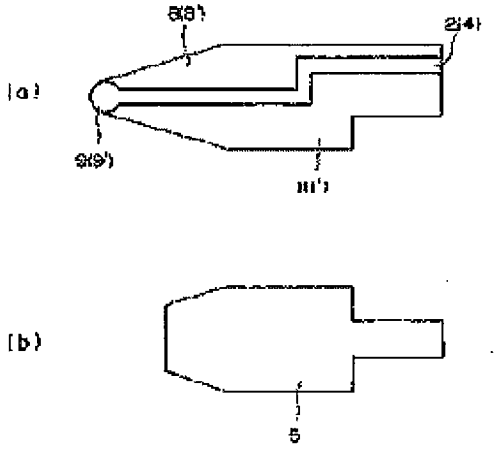


Figure 4

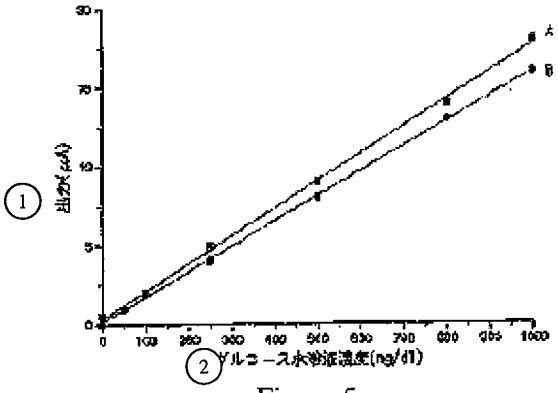


Figure 5

Key: 1 Output (μA)
2 Aqueous Glucose Solution Concentration (mg/dL)

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May 3, 2006

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